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Polymorphisms in the Immunoregulatory Genes are Associated with Hematopoietic Recovery and Increased Susceptibility to Bacterial Infections in Patients with Thalassaemia Major Undergoing Matched Related Hematopoietic Stem Cell Transplantation

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In this study, the impact of polymorphisms in the genes of proinflammatory (IL-β, TNF-α, IL-6, IFN-γ), anti-inflammatory (transforming growth factor [TGF]-β, IL-10, IL-Ra), and other immunoregulatory factors (FcγRIIa, NOS3) along with the conventional risk factors on the rate of hematopoietic recovery and first episodes of bacterial, viral, or invasive fungal infections in 102 patients with β-thalassaemia major who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) with relatively uniform protocols at our center from June 1995 to June 2004 with a minimum follow-up of at least 2 years were studied retrospectively for 180 days after hematopoietic stem cell transplantation (HSCT). Our data show that (1) donor IL-1RN*2/2 (hazard ratio [HR], 2.4; 95% confidence interval [CI], 1.17-5.09; $P = .018$) and *FCγRIIA +4481G/G* genotypes (HR, 3.1; 95% CI, 1.56-6.31; $P = .001$) increased the incidence of bacterial infection; (2) fungal infection was increased in recipients with whose donors had *IFN-γ +874T/T* genotype (HR, 3.8; 95% CI, 1.08-13.62; $P = .037$); (3) time to neutrophil recovery was shorter in splenectomized patients (HR, 3.1; 95% CI, 1.70-5.64; $P < .001$), donors without *IL-10 -1082A*, *-819T*, and *-592A* haplotype (HR, 1.6; 95% CI, 1.02-2.39; $P = .039$), and recipients with *IFN-γ +874A/A* genotype (HR, 1.6; 95% CI, 1.05-2.56; $P = .029$); and (4) time to platelet recovery was shorter in patients with *IL-10 -1082A/A* genotype (HR, 1.8; 95% CI, 1.14-2.68; $P = .010$) and with donors having *TNF-α -308G/G* genotypes (HR, 1.8; 95% CI, 1.06-2.93; $P = .028$). These data suggest that outcome after allogeneic stem cell transplantation could be affected by many factors. The mechanisms by which they bring about such impact needs further evaluation.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the treatment of choice for various malignant [1] and nonmalignant hematological diseases [2]. Infections are a major cause of morbidity and mortality in patients undergoing allo-HSCT despite use of antibiotics and growth factors. Host defenses are profoundly altered due to the conditioning regimen, the cytopenia resulting from it, use of central venous catheters, prolonged administration of immunosuppressive drugs, and graft-versus-host disease (GVHD), if that occurs. All these factors causing the breakdown of the immune system could predispose transplantation recipients to bacterial, fungal, and viral infections during post-HSCT. However, despite having similar risk profiles, only a subset of at-risk individuals has been reported to develop infections. There is strong evidence for polymorphisms in the gene regulatory regions accounting for inter-individual differences in the degree of their expression or their functional ability [3-5].

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Because most of the identified risk factors for infections affect the recipient's immune system, we hypothesize that genetic variation resulting in functional variations within key innate or adaptive immune response elements of the immune system could influence the rate of hematopoietic recovery and susceptibility to infections during post-HSCT. A number of functionally relevant single- nucleotide polymorphisms in genes encoding components of immune system-like inflammatory cytokines (IL-1, tumor necrosis factor [TNF]-α, and IFN-γ) [3], host defense molecules (FcγRIIa, MBL2, and MPO), [4] and immune modulators (IL1Ra) [5] have also been reported to modify the levels of these mediators secreted or expressed, thereby possibly altering the rate of hematopoietic recovery [6] and immune responses resulting in varied susceptibility to bacterial, viral, and fungal infections [7,8]. We have correlated polymorphisms in genes of cytokines, TNF-α, IL-1β, IL-6, IFN-γ, IL10, transforming growth factor-β, and other host defense molecules, FcγRIIa, nitric oxide, and IL1Ra with the time to neutrophil and platelet recovery and incidence of infections in patients with thalassaemia major who underwent matched related allo-HSCT.

PATIENTS AND METHODS

Patients and Eligibility Criteria

One hundred twenty patients with β-thalassaemia major who underwent allo-HSCT at our center from June 1995 to June 2004 were included. Patients for whom DNA samples were unavailable (n = 18) were excluded from the study. All donors were HLA identical sibling (n = 97) or other (n = 5) close family members either father or mother of the recipient. The study was approved by the institutional review board and informed consent was obtained from the parents of all children enrolled in this study.

Endpoint Definitions

Engraftment or neutrophil recovery was defined as the first of 3 consecutive days of an absolute neutrophil count (ANC) of greater than 0.5 × 109/L [9]. Platelet recovery to greater than 20 × 109/L was defined as the day when the first of 2 consecutive platelet counts satisfied the conditions as previously described [10]. Three patients died in the first 21 days after transplantation without having achieved neutrophil recovery and, therefore, were excluded from the engraftment analysis.

Bacterial infections were documented on positive culture from any sites—blood, urine, sputum, pus, abscess, and central venous catheter [11]. Positive blood cultures of common skin contaminants were documented as blood stream infection by performing at least 2 consecutive blood cultures drawn on separate occasions. Urine culture was considered to be significant if the colony count was >100,000/mm^3 or if the colony count was <100,000/mm^3 but considered to be significant if the patient was already on antibiotics. Sputum culture was significant in the presence of pulmonary findings or x-ray infiltrates. The diagnosis of tuberculosis was based on biopsy showing granulomas positive for AFB and/or cultures being positive.

Cytomegalovirus (CMV) infection was defined as 2 consecutive positive DNA-based CMV PCR assays, and CMV disease was defined as the demonstration of CMV in biopsy specimen from different sites of involvement as previously reported [12]. The diagnosis of herpes simplex and zoster was made on clinical grounds, whereas tissue invasion was proven on biopsy and histology. Adenovirus antigen detection was based on ELISA. All fungal infections were documented as probable, possible, and proven fungal infections based on the Centers for Disease Control criteria [13].

Gene Polymorphism Analysis

Genomic DNA was extracted from peripheral blood leukocytes from patients and donors by standard procedures. Previously published protocols for PCR and restriction digestion were used to identify the intron2 (*+3953 T→C*) *IL-1β* polymorphism, promoter region (*-511 C→T*) *IL-1β* polymorphism, and an 86-bp tandem repeat (VNTR) in intron2 of *IL-1RA* [14]. Polymorphisms in the promoter regions of *IL-6* and *TNF-α* gene, respectively, at *-174 G→C* and *-308 G→A* positions and *IL-10* promoter polymorphisms (*-1082 G→A*, *-819 A→T*, *-592 A→C*) constituting 6 different promoter haplotypes were determined by CYTGEN cytokine genotyping trays (One Lambda, Canoga Park, CA) [15]. Both *+869 T→C* (codon 10) and *+915 G→C* (codon 25) polymorphisms in exon1 of *TGF-β* gene and an intron2 polymorphism of *IFN-γ* at position *+874* were also screened using the above-mentioned kits in all the recipient/donor pairs.

Genotyping for *FCγRIIA +4481 A→G* polymorphism was performed by allele-specific restriction enzyme digestion, as described by Jiang et al. [16]. The *G→T* polymorphism in exon7 of *NOS-3* gene (at position 894) in codon 298 was studied using the previously published protocol [17].

Statistical Analysis

Descriptive statistics were calculated for all variables. The predictive effect of recipient and donor gene polymorphisms of proinflammatory (TNF-α, IL-1β, IL-6, and IFN-γ), anti-inflammatory (IL-1Ra, IL-10, and TGF-β) and other host defense molecules, FcγRIIa, nitric oxide were assessed for the outcome of allo-HSCT by Cox proportional

Table 1. Details of Patients, Disease, and Transplantation Protocol (n = 102)

Recipient	No. of Patients 102 (%)
Age, years	
Median (range)	6.5 (1-24)
Sex	
Male	64 (63)
Female	38 (37)
Disease stage	
Class I	8 (8)
Class II	44 (43)
Class III	50 (49)
Splenectomy	16 (16)
Donor	**No. of Patients 102 (%)**
Age, years	
Median (range)	7 (1-42)
Sex	
Male	56 (55)
Female	46 (45)
Female donor to male recipient	32 (31)
Sex-matched transplantations	58 (57)
ABO compatibility	
ABO match	61 (60)
ABO major incompatibility	27 (27)
ABO minor incompatibility	14 (14)
Transplantation	
Conditioning	
Bu (16 mg/kg) Cy (200 mg/kg) + ALG	66 (65)
Bu (14 mg/kg) Cy (160 mg/kg) + ALG	6 (6)
Bu (600 mg/m^2) Cy (200 mg/kg) alone	30 (29)
GVHD prophylaxis	
CSA + short course MTX	102 (100)
Nucleated cell dose (×108/kg)	
Median (range)	4.4 (2.2-9.8)

ABO indicates blood group types; Bu, busulfan; Cy, cyclophosphamide; ALG, anti-lymphocyte globulin; GVHD, graft-versus-host disease; CSA, cyclosporine A; MTX, methotrexate.

hazard model. The clinical variables included recipient/donor age and gender, sex mismatch (in particular, female donor to male recipient), blood group types (ABO) major incompatibility, nucleated cell doses administered, splenectomy performed before transplantation, and the risk category as per Pesaro criteria [18]. Cox proportional hazard model was used to identify the predictive effect of immunogenetic variables in the development of early infections (bacterial, viral, and fungal) at day 180 and time to neutrophil and platelet recovery posttransplantation. Multivariate analyses were done using the forward stepwise procedure to identify the independent predictor of each endpoint. All predictors that were significant ($P < .05$) by univariate analysis were included in multivariate analysis. All tests were 2-sided, and the type 1 error rate was fixed at 0.05. Statistical analyses were performed using the SPSS 11 software package.

RESULTS

Patient and donor characteristics including age, sex, stage of the disease, and details of the transplantation are shown in Table 1.

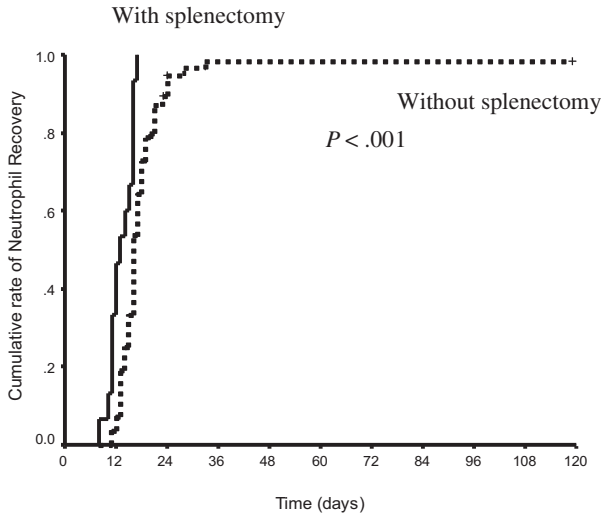

Figure 1. Cumulative rate of neutrophil recovery with respect to splenectomy. Cumulative rate of neutrophil recovery was faster with a median of 13 days in patients who had undergone splenectomy before transplantation compared to 16 days in those who had not ($P < .001$).

Overall Outcome

Of the 102 patients evaluated, 96 (94%) attained an ANC of $>0.5 \times 10^9$/L. The median time to recover a neutrophil count $>0.5 \times 10^9$/L was 16 days (range, 8-33 days). Ninety-one patients (89%) reached a platelet count of $>20 \times 10^9$/L. The median time to achieve an unsupported platelet count $>20 \times 10^9$/L was 28 days (range, 13-154 days). Of the 96 evaluable patients for GVHD, 54 patients (56%) developed acute GVHD grade I to IV.

At day 180 after transplantation, 79 patients (77.45%) had experienced at least 1 episode of documented infection. Infections were documented at various sites as follows: bacterial infections—blood (n = 41), central venous catheter (n = 2), urinary tract (n = 7), and skin (n = 1); viral infections—blood (n = 35), skin (n = 4), oral (n = 4), lung (n = 2), gastrointestinal tract (n = 3), and urinary tract (n = 1); fungal infections—lung (n = 8) and blood (n = 2). Cumulative incidence of at least 1 bacterial, viral, and fungal infection at day 180 was 50% (n = 51), 48% (n = 49), and 9.8% (n = 10), respectively.

Of the 24 patients who died, infections were the major cause for mortality in 9 (37.4%)—bacterial (n = 4; 16.6%), viral (n = 3; 12.5%), and fungal (n = 2; 8.3%).

Engraftment—Recovery of Neutrophil and Platelet Counts

Neutrophil recovery (ANC $>0.5 \times 10^9$/L) was faster with a median of 13 days (range, 8-17 days) in patients who had undergone splenectomy before transplantation compared with 16 days (range, 11-119 days) in those who had not ($P < .001$; Figure 1). Donor IL-10 and recipient *IFN-γ* gene polymorphisms

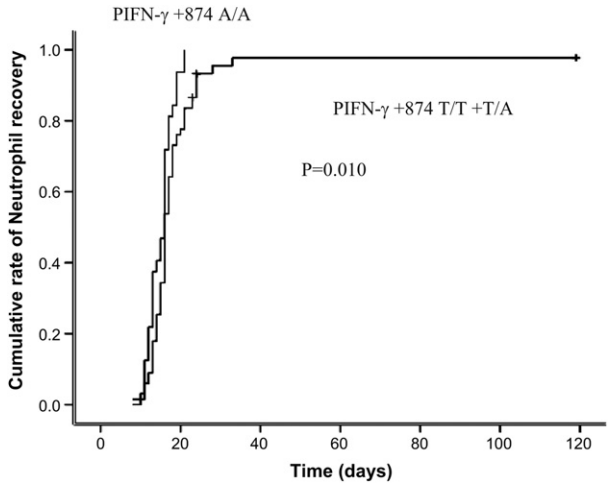

Figure 2. Cumulative rate of neutrophil recovery with respect to *IFN-γ +874* gene polymorphism in recipients. Cumulative rate of neutrophil recovery was faster in recipients with IFN-γ +874 A/A genotype compared to those with IFN-γ +874 T/T +T/A genotypes ($P = .010$). (PIFN-γ - "P" represents patient or recipient.).

influenced the time to neutrophil recovery. Recipients with the *IFN-γ +874T/T+T/A* genotype were associated with a delayed neutrophil recovery (33 days) compared with those with the *IFN-γ +874A/A* genotype (21 days; $P = .010$; Figure 2). Donors with *IL-10 -1082A*, *-819T*, and *-592A* haplotype were associated with a delayed neutrophil recovery (33 days) compared with those without *IL-10 -1082A*, *-819T*, and *-592A* haplotype (24 days; $P = .021$). In multivariate analysis, time to neutrophil recovery was shorter in splenectomized patients (hazard ratio [HR], 3.1; 95% confidence interval [CI], 1.70-5.64; $P < .001$), donors without *IL-10 -1082A*, *-819T*, and *-592A* haplotype (HR, 1.6; 95% CI, 1.02-2.39; $P = .039$), and recipients with *IFN-γ +874A/A* genotype (HR, 1.6; 95% CI, 1.05-2.56; $P = .029$; Table 2).

Time to platelet recovery was shorter in patients with donors having *TNF-α -308G/G* genotype (27 days; range, 13-96 days) compared with those with *TNF-α -308A/A+G/A* genotype (34 days; range, 15-154 days; $P = .035$). Similarly, time to platelet recovery was shorter in patients aged <10 years (28 days; range, 13-96 days; $P = .047$) and those with *IL-10 -1082A/A* genotype (26 days; range, 13-52 days; $P = .012$) compared with patients with age >10 years (32 days; range, 17-154 days) and those with *IL-10 -1082G/G+G/A* genotypes (32 days; range, 15-154 days), respectively. In multivariate analysis, time to platelet recovery was shorter in patients with donors having *TNF-α -308G/G* genotype (HR, 1.8; 95% CI, 1.06-2.93; $P = .028$) and recipients with *IL-10 -1082A/A* genotype (HR, 1.8; 95% CI, 1.14-2.68; $P = .010$; Table 3).

Infections

Donors having *FCγRIIA+4481G/G* (HR, 3.1; 95% CI, 1.56-6.31; $P = .001$) and *IL-1RN*2/2* genotypes (HR, 2.4; 95% CI, 1.17-5.09; $P = .018$) showed an increased risk of bacterial infections by day 180 by multivariate analysis (Table 4A). Conventional risk factors such as acute GVHD and ANC recovery were not found to be associated with the risk of developing bacterial infections. However, when the episodes of bacterial infections were categorized into pre-engraftment and postengraftment periods and included in the multivariate analysis after results were obtained: (1) recipients having *FCγRIIA+4481G/G* genotype (HR, 2.7; 95% CI, 1.04-7.19; $P = .041$); (2) donors having *FCγRIIA+4481G/G* genotype (HR, 2.4; 95% CI, 1.03-5.82; $P = .043$); and (3) pre-engraftment period (neutropenia) increased the risk of bacterial infections (HR, 37.9; 95% CI, 14.58-98.86; $P < .001$; Table 4B).

Among the conventional risk factors, the pre-engraftment period (neutropenia) (HR, 6.1; 95% CI, 2.48-14.94; $P < .001$) and acute GVHD overall grade (HR, 2.1; 95% CI, 1.15-3.89; $P = .017$), grade 2 to 4 (HR, 2.4; 95% CI, 1.36-4.26; $P = .003$), and grade 3 to 4 (HR, 2.7; 95% CI, 1.34-5.28; $P = .005$) increased the risk of developing viral infections. None of the immunoregulatory markers were found to be associated with the risk of developing viral infections. Presence of *IFN-γ +874T/T* (HR, 3.8; 95% CI, 1.08-13.62; $P = .037$) genotype in the donors was found to increase the risk of fungal infections. Among the conventional risk factors, pre-engraftment period (neutropenia) increased the risk of developing fungal infection (HR, 80.6; 95% CI, 13.78-472.05; $P = .001$), and acute

Table 2. Association of Immunoregulatory Gene Polymorphisms in Recipients/Donors and Splenectomy with Neutrophil Recovery

		Neutrophil Recovery	Univariate[a]		Multivariate[a]	
Marker/Variables	Genotypes/Variables	Median Days (Range)	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Splenectomy	Yes	13 (8-17)	3.0 (1.69-5.42)	< .001	3.1 (1.70-5.64)	< .001
	No	16 (11-33)	Reference		Reference	
rIFN-γ +874	A/A	16 (10-21)	1.7 (1.08-2.60)	.021	1.6 (1.05-2.56)	.029
	T/T+T/A	16 (8-33)	Reference		Reference	
dIL-10 -1082A, -819T, -592A haplotype	Without ATA	16 (8-24)	1.6 (1.02-2.38)	.038	1.6 (1.02-2.39)	.039
	With ATA	16 (10-33)	Reference		Reference	

HR indicates hazard ratio; CI, confidence interval.
[a]Cox regression analysis; "r" represents a specific genotype in the recipient, and "d" represents a specific genotype in the donor.

Table 3. Association of Immunoregulatory Gene Polymorphisms in Recipients/Donors and Recipient Age with Platelet Recovery

		Platelet Recovery	Univariate[a]		Multivariate[a]	
Marker/Variables	Outcome/Genotypes	Median Days (Range)	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Recipient age	<10	28 (13-96)	1.7 (0.98-2.81)	.058		NS
	>10	32 (17-154)	Reference			
dTNF-α-308	G/G	27 (13-96)	1.7 (1.02-2.78)	.044	1.8 (1.06-2.93)	.028
	G/A+A/A	34 (15-154)	Reference		Reference	
rIL-10-1082	A/A	26 (13-52)	1.7 (1.09-2.56)	.016	1.8 (1.14-2.68)	.010
	G/G+G/A	32 (15-154)	Reference		Reference	

HR indicates hazard ratio; CI, confidence interval; NS, not significant.
[a]Cox regression analysis; "r" represents a specific genotype in the recipient, and "d" represents a specific genotype in the donor.

GVHD grade 3 to 4 showed a trend for developing fungal infections (HR, 3.8; 95% CI, 0.92-16.08; $P = .065$; Table 5).

Overall Survival

Recipient age >10 (HR, 3.6; 95% CI, 1.54-8.32; $P = .003$) and sex mismatched transplantations (HR, 4.1; 95% CI, 1.46-11.74; $P = .007$) were the conventional factors found to be associated with poor overall survival in this study group. Recipient IL-10 and donor *IFN-γ* gene polymorphisms also influenced the overall survival time. Donors with the *IFN-γ +874T/T* genotype (HR, 3.1; 95% CI, 1.24-7.85; $P = .016$) and recipients with the *IL-10 -819T/T* genotype (HR, 2.9; 95% CI, 1.13-7.54; $P = .027$) were found to be associated with poor overall survival in this cohort (Table 6).

DISCUSSION

Several new findings emerge from these data. Among the conventional factors evaluated, we observed a faster rate of ANC >0.5 × 10^9/L recovery in splenectomized patients, as has been previously reported, where the recovery of peripheral granulocytes (to levels of 200, 500, and 1,000/mm^3) and platelets (levels of 50 and 100 × 10^3/mm^3) were shown to occur rapidly in the splenectomy group compared to the nonsplenectomy group after transplantation [19]. Delayed recovery may also be related to hypersplenism posttransplantation. Splenomegaly in patients with β-thalassaemia might be the cause for the delayed rate of neutrophil recovery in our cohort of patients because excessive sequestration of infused stem cells in the spleen of patients with splenomegaly has been shown [20]. This association between splenectomy and faster ANC recovery in patients with β-thalassaemia undergoing allo-HSCT has not been previously reported. However, splenectomy does not affect overall survival among these patients (data not shown), as reported in our previous study, which had analyzed the impact of pretransplantation splenectomy only in patients with class III thalassaemia [21].

Our data also show that both recipient and donor *IL-10* gene polymorphisms affect hematopoietic recovery. Presence of donor *IL-10 -1082A*, *-819T*, and *-592A* haplotype, which is associated with lower levels of IL-10 and presence of recipient *IL-10 -1082G/G+G/A* genotype associated with higher and intermediate levels [22] correlated with delayed neutrophil and platelet recovery, respectively, in these study patients. IL-10 genotypes contributing to lower levels of IL-10 hampering neutrophil recovery may explain the necessity of the activated donor T cells in the bone marrow innoculum is not only needed to exhibit the GvL effect to prevent relapse but also to improve neutrophil engraftment with their source of cytokines (IL-10) by suppressing the inhibitory cytokines of granulopoiesis (IFN-γ and TNF-α) produced by the residual recipient cells after conditioning [23].

From the observation of recipient IL-10 genotypes (higher levels) impeding platelet recovery, we may

Table 4A. Association of Immunoregulatory Gene Polymorphisms in Recipients and Donors with Bacterial Infections up to Day 180

		Bacterial Infection		Univariate[a]		Multivariate[a]	
Marker	Genotypes	Yes	No	HR (95% CI)	*P* value	HR (95% CI)	*P* value
dIL-1Ra	IL-1RN*2/2	9	4	2.1 (1.02-4.35)	.045	2.4 (1.17-5.09)	.018
	Others	41	47	Reference		Reference	
rFcγRIIA +4481	A/G +G/G	41	26	2.7 (1.31-5.56)	.007		NS
	A/A	9	23	Reference			
dFcγRIIA +4481	A/G+G/G	39	23	2.6 (1.36-4.96)	.004	3.1 (1.56-6.31)	.001
	A/A	12	27	Reference		Reference	

HR indicates hazard ratio; CI, confidence interval; NS, not significant.
[a]Cox regression analysis; "r" represents a specific genotype in the recipient, and "d" represents a specific genotype in the donor.

Table 4B. Association of Immunoregulatory Gene Polymorphisms in Recipients and Donors with Bacterial Infections up to Day 180 with Episode of Bacterial Infections Categorized into Pre-Engraftment and Postengraftment Time Period

		Bacterial Infection		Univariate[a]		Multivariate[a]	
Marker/Variable	Genotypes/Variables	Yes	No	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Time of episode	Pre-engraftment	32	3	21.3 (10.59-42.83)	<.001	37.9 (14.58-98.86)	< .001
	Postengraftment	19	48	Reference		Reference	
dIL-1Ra	IL-1RN*2/2	9	4	2.1 (1.02-4.35)	.045		NS
	Others	41	47	Reference			
rFcγRIIA +4481	A/G+G/G	41	26	2.7 (1.31-5.56)	.007	2.7 (1.04-7.19)	.041
	A/A	9	23	Reference		Reference	
dFcγRIIA +4481	A/G+G/G	39	23	2.6 (1.36-4.96)	.004	2.4 (1.03-5.82)	.043
	A/A	12	27	Reference		Reference	

HR indicates hazard ratio; CI, confidence interval; NS, not significant.
[a]Cox regression analysis; 'r' represents a specific genotype in the recipient and 'd' represents a specific genotype in the donor.

speculate that the residual cells of recipients (T cells) that form the possible source of IL-10 may influence platelet recovery. Previous reports showing the IL-10 role on related suppression of IL-1β [24,25] production, which induces megakaryocyte colony- forming units from murine and human hematopoietic cells [26] could also form the supporting evidence for our observation. Recombinant IL-10 has also been shown to decrease platelet counts and hemoglobin levels when administered to normal healthy adult volunteers [27]. There are also other reports of cytokines produced by lymphocytes and macrophages (IFN-γ and TNF-α) of recipients after bone marrow transplantation having inhibitory effects on hematopoiesis [28] thereby supporting our findings of recipient *IFN-γ +874T/T+T/A* genotypes associated with higher levels of IFN-γ correlating with delayed neutrophil recovery (Table 2).

Although the role of residual cells of recipients being the source of proinflammatory cytokines is evident from several in vivo studies, the recipient cells being the source of IL-10 are speculative. Nevertheless, various results of previous clinical correlations [29] confirm the principle that the recipient response is critical in bone marrow transplantation outcome during the early posttransplantation period.

Our data further show, we believe for the first time, the possible role of *IL-1RN*2/2* and *IFN-γ +874T/T* genotypes and also confirms the role of *FCγRIIA +4481G* allele in infections [8] among patients with β-thalassaemia undergoing allo-HSCT. Donor *IL-1RN*2/2* genotype associated with high serum levels of IL-1Ra [14] and *FCγRIIA +4481G* allele accounting for reduced receptor affinity and specificity for IgGs [4,30] were found to significantly increase the risk of bacterial infections by day 180 (HR, 3.1; 95% CI, 1.56-6.31; $P = .001$; Table 4A). It is possible that polymorphism in *IL-1RA* and *FCγRIIA* genes of the donors make recipients of HSCT susceptible to more bacterial infection during the early posttransplantation period by antagonizing IL-1β produced by host defense cells [5] and decreasing the phagocytic activity [31], respectively. Observation of recipient *FCγRIIA +4481G* allele increasing the risk of bacterial infection during the pre-engraftment period (Table 4B) emphasizes the role of phagocytes of recipient origin in conferring protection against bacterial infection during the early posttransplantation period before engraftment. These results were also consistent with the previous reports [8].

Donor *IFN-γ +874T/T* genotype associated with higher levels of IFN-γ [22] was found to be associated with increased incidence of fungal infections. These results seem to be discrepant from the previous reports showing increased resistance for invasive fungal infections on administration of recombinant IFN-γ [32]. However, no correlation with *IFN-γ* genotypes with incidence of fungal infections has been reported

Table 5. Association of Immunoregulatory Gene Polymorphisms in Recipients and Donors with Fungal Infections up to Day 180 with Episode of Fungal Infections Categorized into Pre-Engraftment and Postengraftment Time Period

		Fungal Infection		Univariate[a]		Multivariate[a]	
Marker/Variable	Genotypes/Variables	Yes	No	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Time of episode	Pre-engraftment	5	4	80.6 (13.78-472.05)	.001		NS
	Postengraftment	5	88	Reference			
aGVHD 3-4	Yes	3	10	3.8 (0.92-16.08)	.065		NS
	No	5	78	Reference			
dIFN-γ +874	T/T	4	12	3.8 (1.08-13.62)	.037		NS
	T/A + A/A	6	80	Reference			

HR indicates hazard ratio; CI, confidence interval; NS, not significant; aGVHD, acute graft-versus-host disease.
[a]Cox regression analysis; "r" represents a specific genotype in the recipient, and "d" represents a specific genotype in the donor.

Table 6. Association of Immunoregulatory Gene Polymorphisms in Recipients and Donors and Overall Survival after HLA Identical HSCT in Patients with β-Thalassemia Major

		Events		Univariate[a]		Multivariate[a]	
Marker	Genotype	Yes	No	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Recipient age	>10	11	13	3.4 (1.52-7.62)	.003	3.6 (1.54-8.32)	.003
	<10	13	65	Reference		Reference	
Donor age	>10	13	21	2.6 (1.16-5.78)	.021		NS
	<10	11	57	Reference			
Sex mismatched	Yes	19	39	3.1 (1.17-8.41)	.023	4.1 (1.46-11.74)	.007
	No	5	39	Reference		Reference	
Splenectomy	Yes	8	8	3.1 (1.34-7.33)	.009		NS
	No	16	70	Reference			
rIL-10-819	T/T	7	7	3.5 (1.43-8.42)	.006	2.9 (1.13-7.54)	.027
	T/C+C/C	17	70	Reference		Reference	
dIFN-γ +874	T/T	7	9	2.7 (1.12-6.54)	.027	3.1 (1.24-7.85)	.016
	T/A+A/A	17	69	Reference		Reference	
dIL-1Ra	IL-1RN*2/2	6	7	3.0 (1.17-7.54)	.022		NS
	Others	17	71	Reference			

HSCT indicates hematopoietic stem cell transplantation; HR, hazard ratio; CI, confidence interval; NS, not significant.
[a]Cox regression analysis; "r" represents a specific genotype in the recipient, and "d" represents a specific genotype in the donor.

previously. Nevertheless, in our study, the donor *IFN-γ +874T/T* genotype was not shown to be an independent risk factor regardless of other conventional risk factors such as neutropenia and acute GVHD grade 3 to 4 for fungal infections (Table 5).

Overall survival was found to be influenced by factors that have been known to be the conventional risk factors for acute GVHD such as increased age of the recipient and sex mismatched transplantations (Table 6). Donor IL-1RN*2/2 genotype associated with bacterial infection did not independently influence overall survival in multivariate analysis. Also splenectomy did not influence overall survival as previously reported in class III thalassaemia patients [21] despite it had shortened the time to ANC recovery in this study group. From this study, we could also envisage that these results were not biased toward worse outcomes in class III patients alone.

Because the polymorphisms in the genes selected for evaluation in this study have also been known to modify the level or functions of the mediators involved in regulating immune response, identification of a genetic polymorphism that influences infectious outcome after HSCT has important implications for pretransplantation and posttransplantation care, and may also have implications for the management of other immune-compromised patients. However, unfortunately, gene polymorphisms in other immune response molecules such as MBL2, MASP2 [7,33], TLR4 [34], IL23R [35], TLR1, TLR6 [36], CXCL10 [37], and plasminogen [38] that have been previously reported to be associated with infections could not be included in this analysis due to insufficiency of DNA in these consecutive patient/donor pairs for genotyping of these immunoregulatory markers.

In our study, regardless of immunoregulatory factors included, various possible risk factors of infections in post-allo-HSCT like neutropenia, acute GVHD, cell dose, and type of conditioning regimen were also tested for the association with the development of infections. However, CMV serostatus that has been previously reported to be associated with an increased risk for infectious complications in patients with post-allo-HSCT was not included in this study owing to high seroprevalence of CMV IgG in the Indian population [39]. Moreover, more than 98% of our recipients are also found to be CMV-positive.

This is the first study to comprehensively evaluate the role of a large number of immunogenetic markers with engraftment, bacterial, viral, and fungal infections in HLA identical sibling transplantation in a cohort of patients with a single nonmalignant genetic disorder. The data obtained enhances our understanding of the pathogenesis of infections and mechanisms of host defenses after allo-HSCT but may also allow for therapeutic decisions to be individualized.

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